Practitioner's Docket No. U 012567-2 *PATENT*

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Suman Preet Singh KHANUJA, et al.
Application No.: 09/487,405 Group No.: 1634
Filed: JANUARY 18, 2000 Examiner: A.K. CHAKRABARTI
For: NOVEL SCREENING METHOD FOR SELECTION OF INSECT TOLERANT PLANTS

BOX RCE
Commissioner for Patents
Washington, D.C. 20231

RESPONSE TO THE OFFICIAL ACTION OF OCTOBER 24, 2001

A Notice of Appeal was filed on April 23, 2002 and a request for continued examination is being filed herewith.

Reconsideration and further examination is respectfully requested in view of the following remarks.

CERTIFICATION UNDER 37 C.F.R. SECTIONS 1.8(a) AND 1.10
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Date: SEPTEMBER 23, 2002

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• Only the date of filing (§ 1.6) will be the date used in a patent term adjustment calculation, although the date on any certificate of mailing or transmission under § 1.8 continues to be taken into account in determining timeliness. See § 1.703(f). Consider "Express Mail Post Office to Addressee" (§ 1.10) or facsimile transmission (§ 1.6(d)) for the reply to be accorded the earliest possible filing date for patent term adjustment calculations.

REMARKS

A declaration of Suman Preet Singh Khanuja, one of the inventors of the present application accompanies this response. As explained in the declaration, the method of the invention relates to a novel and specific procedure targeted to screen, identify and develop insect tolerant plant genotypes or clones through tissue culture as somaclones and simultaneously establishing their molecular distinctiveness through RAPD analysis prior to phenotypic evaluation at *in vitro* stage itself. By using RAPD at this step, only the variant plant are selected and not all the plants that arise from tissue culture.

Field evaluation under natural or artificial insect infestation condition is an essential follow-up step to validate the insect tolerant clones for performance in the field conditions. However, the efficiency and high success rate of the invention is based on the fact that only those clones that are taken for trials at the field level are confirmed insect tolerant at the culture stage. Identifying insect tolerance prior to testing at the field level reduces the time, space and labor of screening.

The Examiner has rejected claims 6-7 and 10-13 as being obvious over the combination of Sondahl and Gilbert further in view of Jones et. al..

The Examiner has rejected claims 6-7 and 9-13 as being obvious over the combination of Sondahl and Gilbert further in view of Jones et. al. further in view of Prajapati et al.

Applicants respectfully traverse these rejections.

As stated in the prior response, the Examiner has relied on impermissible hindsight in making these rejections. As explained in the attached declaration, there is no combination of these references which makes the claimed invention obvious.

On pages 3 and 4 of his declaration, Dr. Khanuja explains the differences between the claimed invention and the Sondahl et al. and explains why the claimed invention is not obvious in view of Sondahl et al. There is no suggestion in Sondahl et al. of identifying the molecular distinctiveness prior to the phenotypic evaluation.

On page 6 of the declaration, Dr. Khanuja describes Gilbert et al. as merely disclosing techniques that are available for the characterization, comparison and analysis of genotypes. The techniques described in Gilbert et al. include RAPD, RFLP, SCAR and ALFP. There is no disclosure or suggestion in Gilbert of using these techniques as is done in the claimed invention.

On page 7 of his declaration, Dr. Khanuja describes the process disclosed in Jones and explains why the process of Jones does not make obvious the method of the invention claimed in this application.

The Kumar paper is also discussed on page 7 and is described as disclosing a tissue process for producing mint plants. This does not cure the deficiencies in the cited combination of references.

Since there is no combination of the references that makes the claimed invention obvious, the disclosure of the insect *Spilarctia obliqua* in Prajapati et al. does not make the claimed invention obvious.

Accordingly, it is respectfully requested that these rejections be withdrawn and this application be passed to issue.

Respectfully submitted,

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IN THE U.S PATENT AND TRADEMARK OFFICE

APPLICANT: Suman Preet Singh Khanuja et al

SERIAL NO: 09/487,405 GROUP: 1634

FILED : January 18,2000

EXAMINER: W. Gary Jones

FOR : A novel screening method for selection of insect tolerant plant

DECLARATION REGARDING NON-OBVIOUS NATURE OF INVENTION

Honorable Commissioner of Patents
Washington, D.C. 20231

Sir,

I, Suman Preet Singh Khanuja, am the first inventor of above-mentioned Patent application and represent all other inventors of the instant Patent Application, do hereby declare as follows:

Our invention relates to a novel and specific procedure targeted to screen, identify and develop insect tolerant plant genotypes or clones involving development of clones through tissue culture as somaclones and simultaneously establishing their molecular distinctiveness through RAPD analysis prior to phenotypic evaluation at *in vitro* stage itself. The RAPD analysis at this step is critical as we select only the sure to be variant plants and not all the plants those arise from tissue culture. This is followed by *in vitro* multiplication and establishing the stability at molecular level through RAPD which is also critical. The variants are transferred to individual culture tubes for forced feeding by insects by releasing actively feeding larvae or nymphs into each culture tube itself. Then the surviving clones (tolerant to insect attack) are *in-vitro* multiplied and rechecked for insect larval non-preference and then field evaluated under natural or artificial insect infestation conditions to confirm the tolerance to insects. The claims are quite clear and supported by experimentation for this novel method. Field evaluation under natural or artificial insect infestation condition is a follow up step to validate the insect tolerant clones for performance in the field conditions. But, the efficiency and

high success rate lies with the fact that only those clones are taken for the trials at field level which are confirmed as insect tolerant at the culture stage itself. This is again **critical as it reduces a lot of time , space and labour for screening. Please find enclosed a reference to support the criticality of the steps in a method (Wilson et al, 2002 , US patent 6,420,630). The first claim reads**

A ***method*** for transforming a line of corn using *Agrobacterium* comprising the steps of:
(a) initiating co-cultivation of an immature embryo from said line with *Agrobacterium* capable of transferring at least one gene to tissue of said line to produce an infected embryo;
(b) applying heat shock treatment during said co-cultivation;
(c) culturing the infected embryo to initiate callus on a medium comprising an antibiotic;
(d) culturing the resulting callus tissue on a medium comprising a selective agent;
(e) selecting transformed callus tissue comprising growing Type II callus; and
(f) regenerating transgenic plants from said Type II callus.

It is understandable that the step (b) in the claim improves the efficiency of transformation of elite corn though the method by applying the heat shock and the rest of the procedure as such was known long before the filing of the patent (Example 3 of the same patent: Effect of Heat Shock Treatment on Integration of DNA). Similarly literatures on co-cultivation with *Agrobacterium* and all other tissue culture methods adopted in the patent are abundantly available in the literature some of which are also mentioned in the same patent. Still the method as a complete system is non-obvious and thus granted. Further the critical step (b) can not change place with other steps in the sequence as described in the method.
Therefore, this invention should be compared as a complete method and not the individual scattered components, which can not be just simply added to reach this procedure.
If compared in isolation the individual components of the procedure are; a. generation of somaclones, b. RAPD analysis, C. screening for insect by feeding with the insect. But, the success of this procedure is in the strategy defined in the embodiment with proper sequence of steps and the tact to utilize the outcome of each step for the next. Please find enclosed the reference Bidney et al (2001) as well as the earlier cited reference **(US patent 6,420,630)** to support the criticality of the sequence involved in a screening method.

The reference (Bidney et al, US patent **6,265,638)** claims (refer claim 12):

A ***method*** for co-transformation of a plant with at least two unique heterologous nucleotide sequences of interest, said ***method*** comprising the steps of a) contacting a tissue from said plant with an *Agrobacterium* strain comprising a helper plasmid and at least two binary vector plasmids that are stably inherited, wherein each of said binary vector plasmids comprises at least one T-DNA region, wherein each of said T-DNA regions comprises one of said unique nucleotide sequences, wherein at least one of said nucleotide sequences comprises at least an expression cassette comprising a plant scorable marker gene;
b) co-cultivating said tissue with said *Agrobacterium*;
c) culturing the tissue in a culture medium comprising an antibiotic capable of inhibiting the growth of *Agrobacterium*;
d) screening the tissue for expression of said plants scorable marker gene; and
e) regenerating a transformed plant from tissue that expresses said plant scorable marker gene.

Here the step b) describes about the co-cultivation with *Agrobacterium*. The next step says to grow the tissue in a medium to score the marker which has been transferred to the plant. If we alter the places of these steps then the method will not be yielding any transformed plant as co-cultivation is a prerequisite for gene transfer from the bacteria to the plant. What will be scored if the marker is not transferred? Similarly this method is constituted of steps if considered individually are obvious, but when taken together is a novel method by it self. This patent (Bidney et al, US patent **6,265,638)** was being granted in the year 2001 and a similar patent **(Wilson et al, 2002 , US patent 6,420,630)** as described earlier was granted in the year 2002. Both describe the transformation methods, which by the given reasoning of the examiner should be obvious. But both the patents are novel if considered as complete system as per the sequence described considering the critical nature of the steps, like our specification.

Examiner cites Sondahl et al

In accordance with the subject of invention, a method is disclosed for the generation of somaclonal variants from an explant of genus Coffee comprising: a) inducing formation of somatic embryos from said explant; b) inducing formation of coffee plants from somatic embryos genesis from said embryos; c) Screening said plants for off parental characteristics. More particularly, these plants are grown under nursery and/or field conditions; and somaclonal variants are recovered by screening the plants for off parental characteristics. In this said citation, characteristics were screened in the field after transferring all the plants to the field. But in our method we transferred the confirmed molecular

variants, further confirmed for the insect tolerant characters through force feeding in tissue culture stage itself and assessed the tolerance level in the field and selected the most tolerant clone avoiding all unnecessary volume, space and labour for thousands of non-variants. The novelty of the system as a method and the reduction in the requirement of labour, time and space ultimately the money needs to be appreciated using the method invented by us. After proposing the method it looks simple and easy but was no way obvious as a method in this holistic manner is not invented earlier. Please find enclosed a reference to support the fact that even a simple method can be non-obvious and patentable. The details are:

Reference (Sano et al, 2002, US patent 6,392,125)

The first claim reads:

1. A ***method*** for producing a transformant of *Coffea arabica*, the ***method*** comprising the steps of: infecting an embryogenic callus of *Coffea arabica* with *Agrobacterium tumefaciens* EHA101 strain that comprises a vector containing an exogenous gene and a gene available for the selection of transformed embryonic callus to produce a transformed embryogenic callus in a medium containing N.sup.6 -[2-isopentenyl]-adenosine, selecting said transformed embryonic callus, forming a somatic embryo from said transformed embryogenic callus and regenerating a transformed Coffea arabica from said somatic embryo.

This is again a similar transformation experiment using similar protocols described earlier for infection (co-cultivation), selection and regeneration through tissue culture method except the plant *Coffea arabica*. This also describes the somatic embryos as described by Sondahl et al (1995). According to the understanding of the examiner the cited patent should have been obvious considering the individual steps like *Agrobacterium* infection (known earlier, Bidney et al, 2001), Selection (Bidney et al, 2001) and regeneration through somatic embryogenesis (Sondahl et al, 1995). But this is not true as the patent is simple and a complete method to get transgenic plants in *Coffea arabica*, and likewise our specification which is novel, non-obvious when considered the steps together in sequence.

Somaclonal variations were known long back (Larkin P J & Scowcroft W R (1981) Somaclonal variation – a novel source of variability from cell culture for plant improvement. Theor Applied Genet. 60:197-214) before the patent of Sondahl et al (1995), still the invention was not obvious. The screening procedure for different characters in the field is written in every book of breeding. The tissue culture regeneration methods are known since the time of Haberlandt (1902) and can be obtained from any standard book of tissue-

culture. The German Botanist is considered to be the father of plant tissue culture who conceived the concept of cell culture in 1902.

Still the patent was granted because of the novelty in the whole system to induce and develop somaclonal variants in coffee. The patent would not have been granted if individual components are compared to other invention in isolation.

Again, we submit that the Examiner would need to deeply go through our invention to understand all details and to understand the concept and essence of the method. If the Examiner understand this fact they must differentiate the efficacy and utility of our method to screen the quality of insect tolerance at the tissue culture stage itself is worth mentionable. The method devised is a rapid and sure protocol to search for the insect tolerant character without taking the whole lot of plants generated through tissue culture for screening through field evaluation. In fact, our method overcomes difficulty of unnecessary large-scale field screening that is required in Sondahl et al.

The insect tolerant characters can be screened as described in the cited patent which is slow, inefficient and requires voluminous land, labour and capital in comparison to our method in mints which is rapid and we screen the plants having changes in DNA and not all the plants in the tissue culture stage itself after which the confirmed plants with the required characters are taken for field evaluation.

Another question can be asked. How can someone say a clone as a somaclone without testing the genetic change that have occurred? The evaluation occurs at the field level morphologically where the contribution of the environment cannot be neglected when all the plants are taken to the field. Further to check the stability, the plants should be scored for the desired characters for a number of generations otherwise, it is a well- known fact that the so-called somaclones generated earlier have reverted back to the original type. **Please find enclosed a reference to support the same (Drew RA, 1995. Application of biotechnology to fruit and nut species. In the sixth conference of the Australian Council on Tree and Nut Crops Inc. Lismore, NSW, Australia. 11-15 September, 1995).**

Sub heading: "Somaclonal Variation and *In Vitro* Selection" which reads:

With the rapid expansion of tissue culture technologies came the observation that genetic off-types were occurring in plants regenerated from somatic cells and this was seen as a novel source of variation for plant. The frequency of off-types occurring in culture varies with species, culture type and number of sub-

cultures and has been attributed to a number of alterations that occur within cultured cells. However, the cause of somaclonal variation is not fully understood, thus it cannot be controlled, and because changes can be **epigenetic and unstable**, its value to applied agriculture has been questioned.

Our method overcomes this aspect of unstability by taking the sure somaclones tested through RAPD at right stage only to the field.

Examiner cites Gilbert et al: for RAPD technique for analysis of characterization of genotypes in corn.

They talk about the techniques available for the characterization, comparison and analysis of genotypes through RAPD, RFLP, SCAR, and AFLP. We would like to inform the Examiner that, apart from these, there are many other techniques like VNTR, STR, Micro satellites, minisatellites and many type of PCR reactions are available to test the plant and animal genotypes. Please refer to the book **"DNA fingerprinting in Plants and Fungi (1995) by Weising K, Nybom, Wolff K, Meyer W. CRC Press"** to support the same. But the question is in what context the techniques are used and to what result? We are not claiming the technique of RAPD but we are claiming a methodology using RAPD at a critical step to achieve genotypes of desired characters in a reduced time frame with fewer requirements of labour, space and money.

Similarly **please find enclosed references on PCR and RAPD (Beck, 2002, US patent 6,358,680)** which claims the method of detecting *Pyrenophora tritici-repentis* using PCR and the patent was granted in the year 2002.

Another example of a patent granted in the year 2001 (**US patent 6,284,466, Benson(2001)**) also teach the PCR method for the detection of polymorphisms in a DNA sequence based on nucleotide differences. Also this patent discloses methods for the identification of individuals, or the species, strain or serotype of an organism using the PCR ***method.*** Still the patent (**US patent 6,358,680)** was being granted because of novelty of use.

Polymerised Chain Reaction (PCR) (Saiki R K, Scharf S, Faloona F, Mullis K B, Horn G T, Erlich H A, and Arnheim N. (1985). Science **230**: 1350-1354.; Saiki R K, Gelfend D H, Stoffel S, Scharf S J, Higuchi R, Horn G T, Mullis K B, and Erlich H A. (1988). Nature (London) **239**: 487- 497.) is the technique used extensively for non hybridisation based fingerprinting and with this discovery the whole technique of DNA fingerprinting was revolutionized. Still after the invention of PCR several PCR based methods were admitted for the grant of patent for the elements of novelty and the context of use.

Examiner cites sturtz et al: *Mentha spicata* plant resistant to wilt and rust

This citation is vague and not relevant to our application. Wilt and Rust are the fungus known long back and man is selecting crop plant varieties for wilt and rust. So, a plant genotype in mint was invented by the inventors of the cited Patent, which does not interfere with our Application. They evaluated the plant of invention for disease tolerance, which is a normal way of evaluation written in any standard plant pathology textbook. Whereas, in the present invention, a sequence of steps are arrived at, in a particular manner to achieve the required results .

Examiner cites Jones et al: talks about forced feeding in pentatomid pests.

Examiner should understand the sequence. First 654 soyabean lines were field-tested and 27 tolerant lines were selected which were subjected to field cage test in the field and green house. In our method, we subjected thousands of plants to RAPD analysis without the need to grow them in field to isolate somaclones, different in their DNA profile and subjected to forced feeding again at the tissue culture stage itself (not the field or cage; please note) to isolate tolerant plants and these were taken to the field afterwards. So this reference does not interfere with our Application.

Examiner cites Kumar et al: talks about using internodal segment of mint plant for *in vitro* culture.

This does not interfere with our invention since we have cited our paper (A.K. Shasany, S.P.S. Khanuja, S Dhawn, U. Yadav, S. Sharma, S.Kumar, High regenerative nature of *Mentha arvensis* internodes. Journal of Biosciences 23 (1998) 641-646) to develop the method of our invention. Also, Kumar et al has obtained the patent for regeneration from internodal tissue. The Patent only describes the technique of tissue culture and does not deal with any method to develop the insect tolerant plant as we have invented which cannot be even though of by the knowledge of cited patent.

Our method is a systematic, holistic procedure to generate genetically different insect tolerant plants and should be followed sequentially as described by us, which will save time, space, labour and money. Definitely our invention is novel, non-obvious and holistic and has commercial application and should be granted the Patent.

Further, there is no direct interference by cited arts. One citation does not make reference or directs or even imply reading other citation. Examiner has simply split methodology into 4/5 parts and has cited one prior art for each one of

them and has tried to reject claims on the basis of obviousness. In nutshell, there is no trail for citations i.e., no sequence or serially established.

Further, to raise the objection of obviousness, there has to be a logical link between one citation to another citation, and such link has to be found in the citation itself. In the absence of such logical link inherent in the citation, one cannot create such link because this will be possible only after this invention is revealed.

Our methodology is developed after much trial and error, and after considerable involvement of human efforts.

In fact, the various citations referred by the Examiner can not be technically linked to arrive at insect tolerant plants produced by the methodology employed in the present invention.

I hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United State Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 26 August 2002

Suman Preet Singh Khanuja

Suman Preet Singh Khanuja